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Relationship between crown like structures and body mass index in breast cancer

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RELATIONSHIP BETWEEN CROWN LIKE STRUCTURES AND BODY MASS INDEX IN BREAST CANCER

By

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ABSTRACT

Introduction

Breast cancer (BC) is the most common malignancy in women worldwide, including Kenya. Globally, increasing incidences of BC have paralleled those of obesity. The mechanism by which obesity contributes to BC is still an active area of research but white adipose tissue inflammation characterised by crown like structures (CLS), which are macrophages surrounding dying adipocytes, is considered an important contributor.

Objectives

The primary objective was to determine the association between CLS and body mass index (BMI) in BC patients. The secondary objectives were to determine the association of CLS with BC risk factors specifically, age, menopausal status, subtypes of BC and overall survival among BC patients.

Methodology

Surrounding non-tumour breast tissue from 180 BC surgical specimens were selected and stained immunohistochemically with CD68 to detect CLS. Demographic and clinical data was collected from medical records. Comparison between CLS presence and BMI categories with the other variables was determined based on Fishers Exact test and Kruskal Wallis test. Multivariable logistic regression modelling was used to test the association between CLS presence and BMI categories. Models were adjusted for age, ER status and menopause as potential confounders. A p value of less than 0.05 was considered statistically significant.

Results

In multivariable models, obese patients were approximately two-fold more likely to have CLS in their tissues than normal weight patients (Odds ratio (95% CI) = 2.03 (0.68-6.02)) but differences were not statistically significant (p = 0.204). BC risk factors specifically, age, menopausal status, subtypes of BC and overall survival among BC patients were also not associated with CLS presence.

Conclusion

The findings were suggestive of higher CLS prevalence in surrounding non-tumour breast adipose tissue of obese than normal weight women. Observed differences were, however, not statistically significant, which could be due to the low prevalence of CLS in this population, the relatively small sample size, or due to inherent tumour characteristics within this population.

Recommendations

Due to its low prevalence, CLS may not be the most ideal marker for white adipose tissue inflammation and further studies looking into macrophage density and different types of macrophages within surrounding breast tissue are recommended.

LIST OF ABBREVIATIONS

AKUHN	Aga Khan University Hospital Nairobi
BAT	Brown adipose tissue
BCT	Breast conservation treatment
BC	Breast cancer
BMI	Body Mass Index
CD68	Cluster of Differentiation 68
CK5/6	Cytokeratin 5/6
CLS	Crown like structures in breast adipose tissue
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
H/E	Haematoxylin and eosin
HER2	Human Epidermal Growth Factor 2
HIC	High income countries
LIC	Low income countries
NFkB	Nuclear Factor kappa-light-chain-enhancer of activated B cell
PR	Progesterone Receptor
WC	Waist circumference
WAT	White adipose tissue

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DECLARATION

I declare this dissertation does not include without acknowledgement any material formerly submitted for a degree or diploma in any university. Information derived from literature has been acknowledged within the text and a list of references provided.

(Signature of candidate)

18th April 2023

Date

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CHAPTER1: INTRODUCTION

1.1 Background

Obesity is one of the few modifiable risk factors of BC which can be addressed through risk reducing interventions (Harbeck et al., 2019). Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue, that impairs health (JS, 1988). While there are multiple measures of obesity, body mass index (BMI) is the most easily obtained and the most commonly measured. In most studies of European ancestry, a BMI of 30kg/m^2 or greater is used to define obesity although this has been suggested to be overestimated in African populations (Heymsfield, Peterson, Thomas, Heo, & Schuna, 2016; WHO, 2000). African American men and women were seen to have less visceral adipose tissue than Hispanic and white men and women for the same BMI and waist circumference (Camhi et al., 2011; Carroll et al., 2008).

BMI, the most commonly used measure, is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). It classifies adults based on their score as either underweight (<18.5), normal (18.5-24.9), overweight (25-<30) or obese (>30). BMI is an indirect measure which does not quantify the amount of adipose tissue, its distribution and status in the body (WHO, 2000). Globally, 1.9 billion adults were overweight in 2016 with 650 million of these being obese (Organisation, 2021). Estimates from Kenya show that 20.5% and 9.1% of women aged 15-49 years are overweight and obese respectively(Mkuu, Epnere, & Chowdhury, 2018). A more recent study from Kenya published in 2021 estimated the mean BMI to be 23.38 (SE=0.22) with 28.08% of the population being overweight/obese(n= 4340 adults, 49.59% women) (Rahma Mkuu, 2021).

The association between BC and increased BMI has been studied extensively in high income countries (HIC) (Neuhouser, Aragaki, et al., 2015; Reeves et al., 2007; White, Nichols, Bradshaw, & Sandler, 2015). Studies done among Caucasian population show that postmenopausal obese (BMI >30kg/m2) women are more at risk for estrogen receptor (ER) positive BC compared to those with normal BMI (Bhardwaj et al., 2019; Neuhouser, Chlebowski, & Anderson, 2015). A study by Blair et al showed that women who presented with tumors greater than 5 cm were more likely to have a BMI > 25kg/m^2 (22.1%, n=859) compared to those with normal BMI (9%, n=859) (Blair, Wiggins, Nibbe, Storlie, & Prossnitz, 2019). Obese women also had a 1.7-fold increased risk of stage 3 or 4 disease from the same study (Blair et

al., 2019). A recent local study by Sayed et al showed that 68.7% of Kenyan BC patients(n=823) had a BMI >25kg/m^2 (Sayed et al., 2018).

Obesity has been shown to increase white adipose tissue inflammation in the breast. Crown like structures in the breast (CLS) are histological markers of this inflammation. Prevalence of breast adipose tissue inflammation has been seen to increase with increase in BMI (34% in overweight, 57% in class I obesity and 65% in class II-III obesity P for trend < 0.01.) (Greenlee et al., 2019). Another study by Vyasse et al found that overweight and obese patients have 3.2 times and 6.9 times higher odds ratio of CLS respectively compared with normal patients (Vaysse et al., 2017). Studies by Koru Sengul et al and Iyengar et al show that CLS are also associated with poor prognosis with reduced distant free survival, shortened disease specific survival and overall survival in BC (Iyengar, Zhou, et al., 2016; Koru-Sengul et al., 2016).

African patients present with BC earlier, at a more advanced stage and with more aggressive BC subtypes (Lukong, Ogunbolude, & Kamdem, 2017). Given this distinct BC profile among African patients and the rise in obesity globally including Africa (Adeboye, Bermano, & Rolland, 2012; Organisation, 2021), studies to confirm and understand the obesity-cancer relationship in this population are needed so as to provide targeted prevention strategies and interventions.

1.2 Problem Statement

The link between obesity and BC has been studied extensively in high income countries (Neuhouser, Aragaki, et al., 2015; Reeves et al., 2007; White et al., 2015). Studies have shown that white adipose tissue inflammation characterized by CLS is an important contributor (Faria et al., 2020; Iyengar, Gucalp, Dannenberg, & Hudis, 2016). Prevalence of CLS has been noted to increase with increase in BMI in different populations but there is scant literature within the Kenyan population (Greenlee et al., 2019; Maliniak et al., 2020; Zhao, Sun, Ye, Zhang, & Yao, 2020).

1.3 Literature Review

1.3.1 Breast Cancer Epidemiology

Breast cancer (BC) is the most common malignancy in women including in Kenya (Harbeck et al., 2019) with an estimated 2.3 million new cases globally in 2020(Observatory, 2020; Sung, Ferlay, Siegel, Jemal, & Bray, 2021). Incidence rates are higher in high income countries (HIC) compared to low middle income countries (LMIC) and this can be attributed to a longstanding higher prevalence of reproductive and hormonal risk factors and lifestyle risk factors, as well as increased detection through organized or opportunistic mammographic screening (Garrow, 1988). However, mortality rates are 17% higher in LMIC compared with HIC (15.0 and 12.8 respectively) (Garrow, 1988). In Africa the mortality rates vary with Western Africa having the highest and Southern Africa having the lowest (22.3 and 15.7 per 100,00 respectively) (Lei, Zheng, Zhang, Zhou, & Wei, 2021). In Kenya the incidence rate is estimated to be 16.1% with a mortality rate of 11.5% (Observatory, 2020). Also, various studies have shown that African and African American patients present with BC at an earlier age compared to White women (Abdulrahman & Rahman, 2012; Aziz et al., 1999). The situation in Kenya is similar with majority (53.9%) of Kenyan BC patients being younger than 50 years (Sayed et al., 2018).

BC is a heterogeneous disease at the morphological and molecular level (Harbeck et al., 2019). The WHO lists up to 21 histological types, with invasive carcinoma of no special type being the most common worldwide (Michael Thun, 2017).

The rest are grouped as special types due to certain morphologic features. At the molecular level BC is classified into 4 intrinsic subtypes; luminal A, luminal B, HER-2 enriched, basal like subtypes (Perou et al., 2000). One can approximate BC molecular subtypes by using an immunohistochemistry panel comprising of ER, PR, HER2, Ki67, CK5/6, EGFR (Schnitt, 2010).

A study by Sayed et al showed that 70.2% of BC in Kenya is Luminal A or B, 10.6% HER2 enriched, and 19.2% were triple negative (ER, PR and HER2 negative) (Sayed et al., 2018). Although early BC which is localized to the breast and axillary lymph nodes is curable in 70-80% of patients, mortality rates are still high in low income countries where patients present with advanced stage disease and/or metastases (Harbeck et al., 2019).

1.3.2 White Adipose Tissue Inflammation and Breast Cancer (BC)

The mechanism by which obesity contributes to BC is still an active area of research and white adipose tissue inflammation represents one of the ways (Calle & Kaaks, 2004; Iyengar, Gucalp, et al., 2016). Adipose tissue can be grouped into white, brown or pink, each with different physiological properties. White adipose tissue (WAT) is the most abundant form in adults, and functions to store energy primarily and is increased in obesity due to excess energy intake. It is found in visceral and subcutaneous location (Cinti, 2018; Correa, Heyn, & Magalhaes, 2019). Brown adipose tissue (BAT) has a thermogenic function and is found mainly in newborns and infants. In adults it is limited to certain areas such as the interscapular region, neck and around major vessels (Correa et al., 2019; Frontini & Cinti, 2010). Both WAT and BAT are interconvertible to so as to meet physiological needs (Cinti, 2018). Pink adipocytes are found within the breast in pregnant and lactating women due to increased milk secretion (Correa et al., 2019).

In obesity there is adipose tissue hypertrophy and hyperplasia. As breast white adipose tissue expands, there is reduced blood supply, hypoxia and adipocyte death. The necrotic adipocyte releases free fatty acids and chemokines which attract bone marrow derived macrophages and causes them to proliferate and polarize to a pro-inflammatory phenotype (Faria et al., 2020; Iyengar, Gucalp, et al., 2016). The macrophages surround the necrotic adipocyte forming crown like structures (Figure 1) (Maliniak et al., 2020; Morris et al., 2011).

The activated macrophages phagocytose the fat and secrete cytokines that maintain the inflammatory state. They also produce reactive oxygen species and reactive nitrogen species which are mutagenic (Berger, 2017). There is also increased expression of the NFkB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway which promotes synthesis of various cytokines (Zhu et al., 2020). Aromatase expression and activity is also increased within the adipose tissue resulting to elevated estrogen (Morris et al., 2011) (Figure 2). All these contribute to the development and progression of BC (Faria et al., 2020; Iyengar, Gucalp, et al., 2016).

Crown like structures in the white adipose tissue of the breast (CLS) are histological markers of this inflammation and increased aromatase activity (Berger, 2017). The CLS are more frequent in obese individuals and the association is similar between Caucasians and African American BC patients (Maliniak et al., 2020). They are also associated with a poorer prognosis in BC (Morris et al., 2011). Various studies have shown that CLS increase with BMI both in mice (Cinti et al.,

2005) and human subjects (Faria et al., 2020; Greenlee et al., 2019; Zhao et al., 2020). They are associated with reduced survival and shortened distant recurrence free survival in those who develop metastases in BC (Iyengar, Zhou, et al., 2016).

CLS has been positively associated with BMI in BC patients among other populations such as Taiwanese, Hispanic/Latina, Chinese, Caucasians and African American (Greenlee et al., 2019; Iyengar et al., 2018; Maliniak et al., 2020; Zhao et al., 2020). Data among African population is currently lacking. CLS are seen more frequently in postmenopausal obese women and hormone receptor positive BC. This is explained by the increased expression and activation of aromatase by white adipose tissue inflammation (Iyengar et al., 2015; Morris et al., 2011).

CLS have also been seen in adipose tissue in other malignancies such as oral squamous cancer (Iyengar, Ghossein, et al., 2016), endometrial cancer (Moukarzel et al., 2022) and prostate cancer (Gucalp et al., 2017) which strongly suggests the role of adipose tissue inflammation in cancer pathogenesis. In both malignancies, white adipose tissue inflammation was positively associated with higher BMI. The presence of CLS in tongue fat is associated with reduced disease-specific and overall survival in patients with early-stage tongue cancer (Iyengar, Ghossein, et al., 2016). In prostatic cancer, peri-prostatic adipose tissue inflammation was seen in 49.7% (n=169) of patients and was associated with higher BMI and Gleason grade group 4/5 tumors (Gucalp et al., 2017).

White adipose tissue inflammation plays a significant role in BC and this study aims to explore the relationship between CLS, BMI and BC.

1.4 Study Justification

BC, the most common malignancy among women, occurs at an earlier age, advanced stage and with more aggressive subtypes in African and African American women (Koru-Sengul et al., 2016; Lukong et al., 2017). BC patients are also more likely to have higher BMI(Blair et al., 2019). There is evidence that breast white adipose tissue inflammation contributes to BC development and progression (Berger, 2017; Zhu et al., 2020). The association though complex is of significance. It will aid in understanding the BC-obesity pathway and provide targeted interventions for this population.

There are limited data on obesity measures in the Kenyan population and we aim to determine associations with histologic measures (CLS) in breast tissues, compare results with other studies elsewhere and provide an understanding of the obesity-cancer relationship in our population. CLS could augment BMI and other obesity measures by providing a tissue specific status of adiposity which will be more consistent in detecting high risk individuals who could benefit from targeted treatment (Iyengar, Gucalp, et al., 2016).

Given the global rise of obesity (Organisation, 2021) it is important to focus on the mechanisms that lead to BC in this population in order to provide targeted prevention and treatment options. Furthermore, success in this field could also be extended to other malignancies that are associated with obesity.

1.5 Research Question

What is the association between crown like structures in white adipose tissue with BMI in women with BC diagnosed at AKUHN?

1.6 Study Objectives

Main Objective:

- To determine the association between crown like structures and BMI in BC

Secondary objectives:

- To determine the association of crown like structures with epidemiological factors specifically, age, menopausal status, subtypes of BC and overall survival among BC patients
- To carry out a comparative analysis of BMI, and other measures of obesity (pictographs and waist circumference) as body fat measures within a subset of cases diagnosed for which this information is available.

CHAPTER 2: METHODOLOGY

2.1 Study Design

This was a retrospective descriptive study

2.2 Study Location

This study was conducted at the Aga Khan University Hospital Nairobi, Pathology Department.

2.3 Study Population

The study population comprised of consecutive BC mastectomy and breast conservation treatment (BCT) specimens received at the Pathology Department AKUHN during the period January 2012 to December 2021.

2.3.1 Inclusion Criteria

Mastectomy and BCT specimens that met the following criteria were selected:

- Available relevant clinical data
- No prior neoadjuvant chemotherapy or radiotherapy given for BC or any other malignancy
- Available formalin fixed paraffin embedded sample of surrounding normal breast tissue in patients diagnosed with BC.

2.3.2 Exclusion Criteria

- Patients with core biopsies only

2.4 Sample Size

This was a retrospective study and we intended to use all the samples available from 2012 – 2021. The estimated sample size for this study was 240 samples.

2.5 Methods

2.5.1 Sample Selection

Breast cancer (BC) cases diagnosed between 2012 and 2021 were identified in CARE pathology database. Specimens which met the eligibility criteria were selected. Clinical and demographic data were obtained from CARE and patient files including: age, menopausal status, weight, height, BMI and tumour characteristics (stage, grade, tumour size and hormone receptor status) and recorded in data sheets (Appendix 1). Waist circumference, hip circumference and pictographs(Appendix 2) were obtained from records of cases diagnosed between 2019 to 2021

Menopausal status was categorized as pre-menopausal and post-menopausal as per clinical notes. BMI was calculated from pre-surgical height and weight and classified as per the WHO definitions: underweight BMI<18.5 kg/m^2, normal weight BMI 18.5- 24.9 kg/m^2, overweight BMI 25-29.9kg/m^2 and obese BMI>29.9 kg/m^2 (WHO, 2000). Tumours were reported as estrogen or progesterone positive if nuclear positivity was >1% on immunohistochemistry as per CAP protocols (College of American Pathologists). HER2 status was determined on IHC and reported as negative, equivocal or positive depending on the membranous staining of the tumour cells as per CAP protocol (College of American Pathologists).

For each eligible BC specimen formalin fixed paraffin embedded block representing surrounding breast tissue was retrieved from the archive at Aga Khan University Hospital Nairobi Pathology Laboratory. Surrounding benign breast tissue was taken as per the standardized method that was implemented by the histology section in January 2018. It comprises benign tissue that is 2cm (peri-tumoral) or 5cm (distant) from the tumour. For cases that were diagnosed earlier than January 2018, the margins that were 2-5cm away from tumour were used instead.

Haematoxylin and Eosin (H/E) sections were cut at 4µm and examined to ensure they contained representative benign breast tissue and had no tumour foci, fat necrosis or inflammation. From this, the best block with adequate, that is 75%, white adipose tissue was selected.

2.5.2 Laboratory Analysis

Sections from the selected FFPE blocks were stained for CD68 (a marker used to identify macrophages) consistent with previously established methods(Greenlee et al., 2019; Maliniak et al., 2020) using immunohistochemistry to evaluate presence of CLS. The stained H/E and CD68 slides were scanned at NCI/NIH laboratory using a whole slide imaging scanner and analysed on the image analysis system HALO (Halo, Indica Labs, Albuquerque, NM).

Total white adipose tissue area was measured in cm^2 on HALO. CLS were identified within the adipose tissue, annotated and manually counted by the investigators on the digital images. Encirclement of at least 50% of the adipocyte by CD68+ cells was required to qualify as CLS (Maliniak et al., 2020). (Figure 1 and 2).

CLS density was given as number of CLS per cm^2 of adipose tissue area (CLS/cm^2). The median was used as the cut-off point to distinguish between low and high CLS density. The images were initially reviewed first by the resident and then together with the supervisor. The reviewers were blinded to the clinicopathological data at the time of CLS scoring.

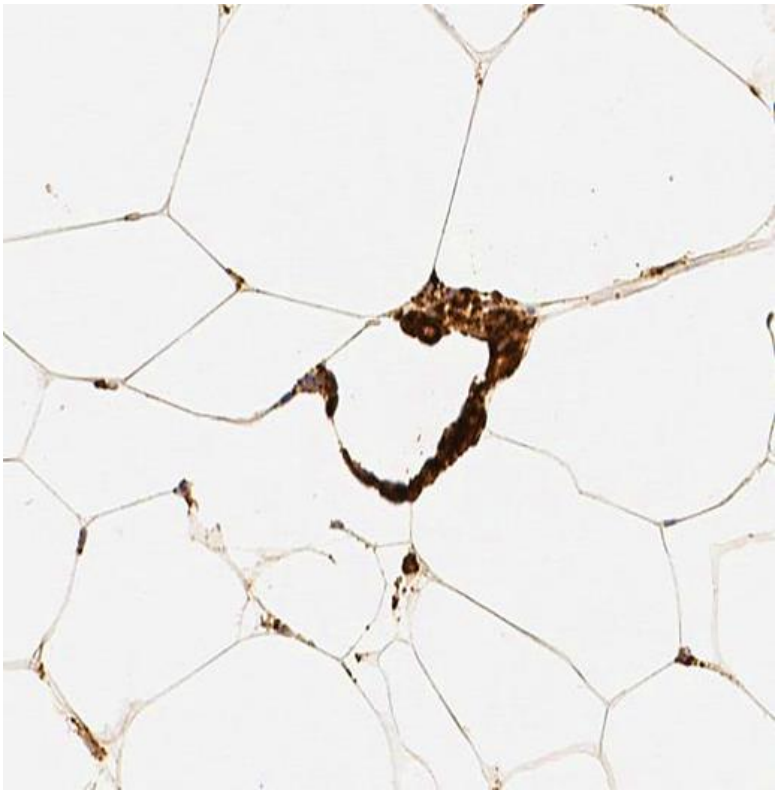

Figure 1: Representative micrograph of a CLS with 50% encirclement of adipocyte with CD68 positive macrophages

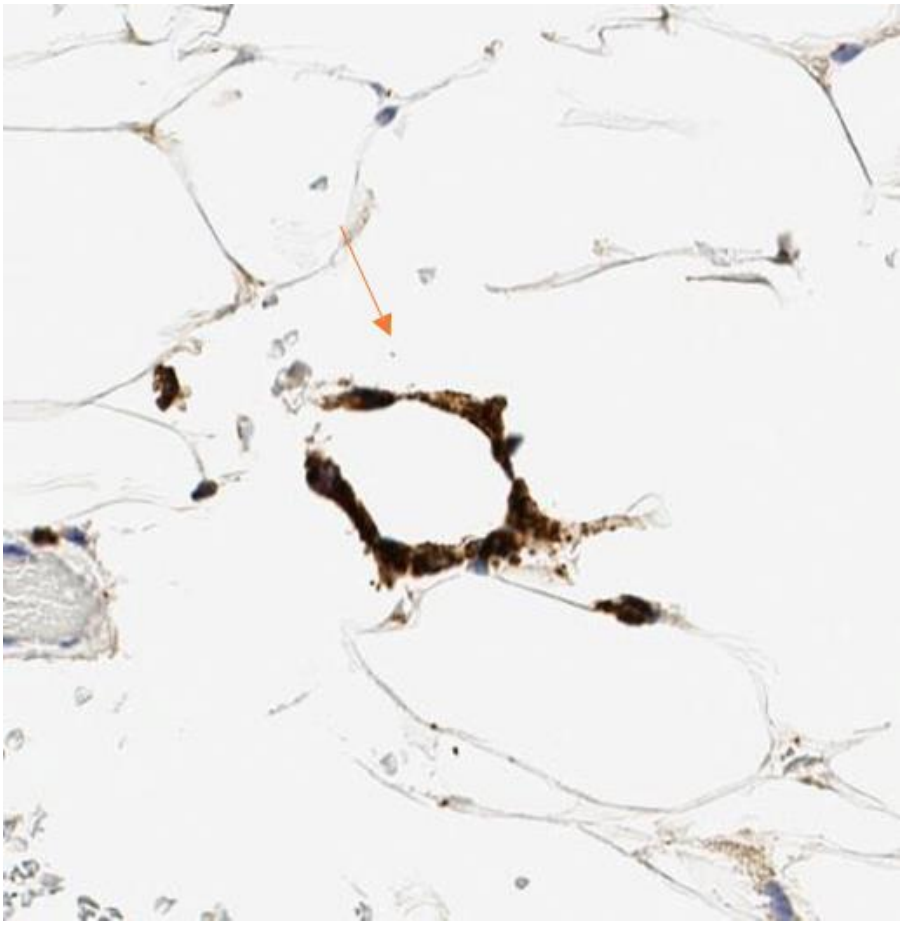

Figure 2: Representative micrograph of a CLS with 100% encirclement of adipocyte with CD68 positive macrophages

2.6 Data Management and Statistical Analysis

Summary statistics were used as frequencies and percentages for categorical variables and means and standard deviations (SD) or medians and interquartile ranges (IQR) for continuous data. Comparison between CLS group (absent vs present) and BMI group (normal vs overweight vs obese) with the other variables was determined based on Fishers Exact test for categorical data and Kruskal Wallis test for continuous data. Multivariable logistic regression modelling was used to test the association between CLS presence and BMI categories.

Models were adjusted for age, ER status and menopause as potential confounders. A p value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS (IBM Version20).

ETHICAL CONSIDERATIONS

The proposal was submitted to the AKU Institutional Scientific and Research Ethics Committee before commencement of the study. An ethical waiver was granted as only archival tissues were used and neither human subjects nor personal identifiers were used for the purposes of this retrospective study. No diagnostic changes arose during this study.

Access to study data was controlled by the principal investigator.

CHAPTER 3: RESULTS

3.1 Study Population Characteristics

258 eligible BC specimens were identified. 57 lacked BMI data. Upon microscopic examination 21 had tumour within the surrounding breast tissue and were excluded leaving 180 for final analysis. This number was consistent with other studies previously.(Greenlee et al., 2019; Zhao et al., 2020)

Mean age at BC diagnosis was 53 years with a mean BMI of 29.9kg/m2. 15.6% had normal BMI, 36.1% are overweight and 48.3% are obese. (Figure 3)

60.8% were postmenopausal. Mean tumour size was 3.8cm. 82.2% had invasive ductal carcinoma and 66.3% were Luminal subtype. Most common histologic grade was grade 2(51.4%). (Table 1 and 2)

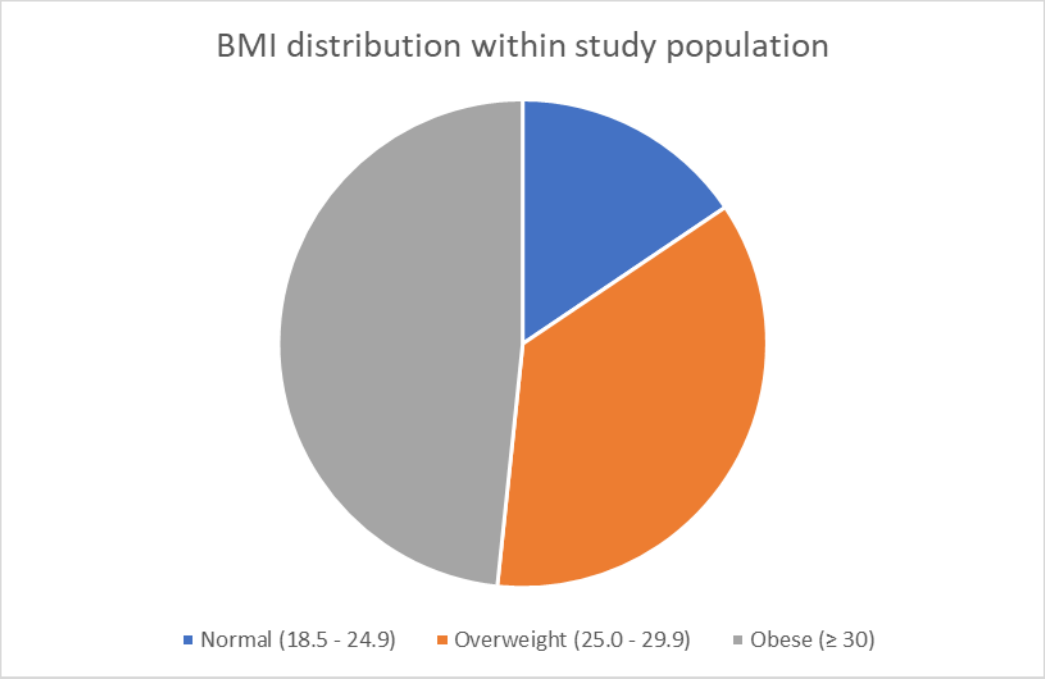

Figure 3: Pie chart showing distribution of BMI within study population

3.2 Association between CLS presence and BMI

CLS were present in 21.67% of cases. The prevalence increased with BMI as 7.7% were identified in normal weight, 33.33% in overweight and 59.0% in obese (Figure 4). CLS density was determined as high or low. Median CLS density was 1.358 CLS/cm^2. (Table 3 and 4)

In multivariable logistic regression models, obese patients were approximately two-fold more likely to have CLS in their tissues than normal weight patients (Odds ratio (95% CI) = 2.03 (0.68-6.02)) but differences were not statistically significant (p = 0.204). BC risk factors specifically, age, menopausal status, subtypes of BC and overall survival among BC patients were also not statistically significantly associated with CLS presence (Table 5)

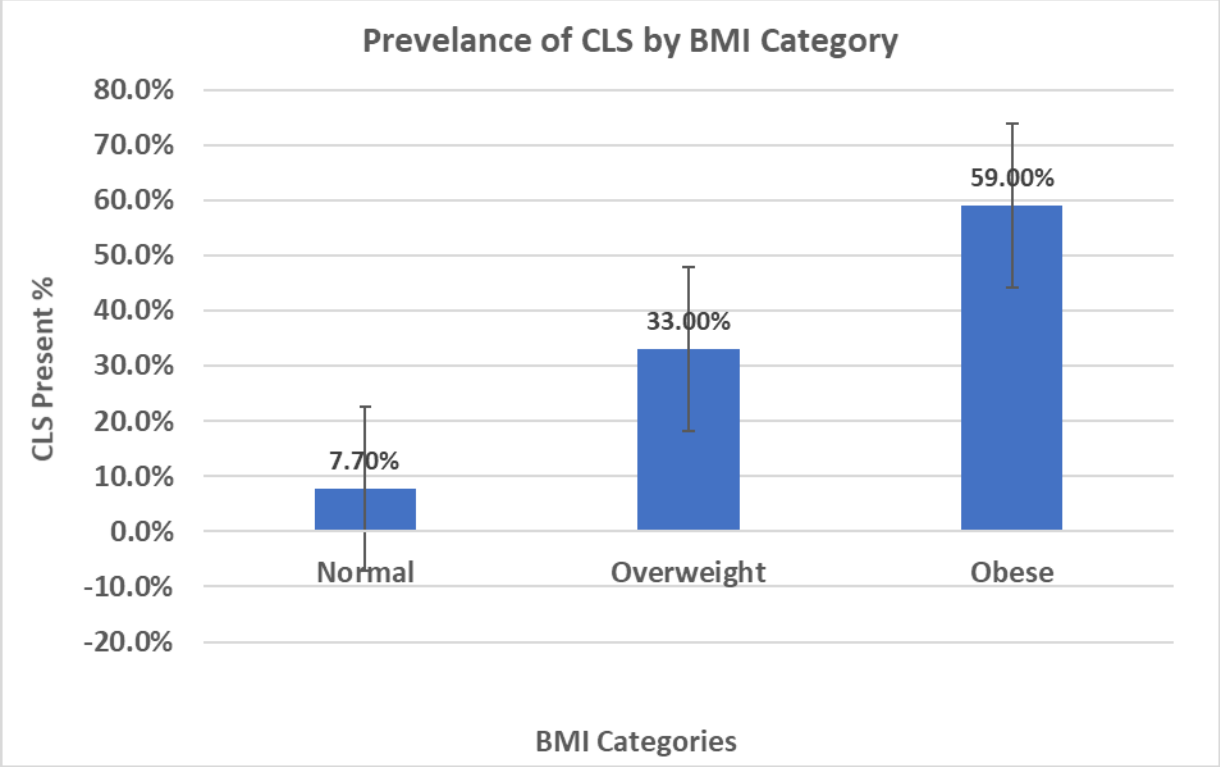

Figure 4: Graph showing prevalence of CLS by BMI category

3.3 Association between CLS and clinical and demographic characteristics

CLS were more common in post-menopausal patients (65.8%). Most patients with CLS had stage 2 disease (71.1%). (Table 6) BC risk factors such as age (p=0.313), menopause status (p=0.669), tumour subtypes and overall survival (p=1.000) among the BC patients were not statistically significantly associated with CLS presence. Due to limited data a comparative analysis between BMI, pictographs, waist circumference and hip circumference could not be efficiently carried out.

Table 1: Clinical and demographic characteristics of study population

Characteristic	Mean[SD]
Age (years) (mean [SD])	52.7 [11.4]
BMI (kg/m2)(mean [SD])	29.9 [5.2]
Waist Circumference (n=50)	53.7 [27.0]
Hip Circumference (n=46)	60.1 [28.5]
Waist Hip Ratio (n=46)	0.94 [0.30]
Tumor size (n=172)	3.8 [2.3]

Table 2: Clinical and demographic characteristics of study population

Characteristic		N	%
BMI Categories	Normal (18.5 – 24.9)	28	15.6%
	Overweight (25.0 – 29.9)	65	36.1%
	Obese (≥ 30)	87	48.3%
Histologic Type	Invasive ductal carcinoma	148	82.2%
	Invasive lobular carcinoma	7	3.9%
	DCIS	5	2.8%
	Metaplastic carcinoma	5	2.8%
	Invasive carcinoma with mixed ductal / lobular	4	2.2%
	Invasive mucinous carcinoma	4	2.2%
	Invasive micropapillary carcinoma	3	1.7%
	Others	4	1.1%
Histologic Grade (n=173)	1	6	3.5%
	2	89	51.4%
	3	78	45.1%
ER	Positive	125	71.4%
	Negative	50	28.6%
PR	Positive	114	65.1%
	Negative	61	34.9%
HER2	Positive	28	16.0%
	Negative	134	76.6%
	Equivocal	9	5.1%
	DCIS, not graded	4	2.3%

Table 2: cont'd…

Characteristic	Characteristic	N	%
Molecular	Triple Positive	5	2.9%
	Triple Negative	17	9.7%
	Core-Basal	17	9.7%
	DCIS, not graded	5	2.9%
	HER-2 Enriched	11	6.3%
	HER-2 Equivocal	2	1.1%
	Luminal	116	66.3%
	Not Classified	2	1.1%
Menopause Status (n=171)	Pre-Menopausal	66	38.6%
	Peri-Menopausal	1	0.6%
	Post-Menopausal	104	60.8%
Survival Status (n=47)	alive and metastases	4	8.5%
	Alive and well	20	42.6%
	alive with treatment	14	29.8%
	Alive and bedridden	1	2.1%
	Alive and taking herbs	1	2.1%
	alive with metastases or recurrence	2	4.3%
	Alive with multiple metastases	1	2.1%
	Alive with recurrence	3	6.4%
	died due to metastases	1	2.1%
Stage (n=170)	1	29	17.1%
	2	109	64.1%
	3	27	15.9%
	4	5	2.9%
Pictograph [1](n=65)	2	5	7.7%
	3	13	20.0%
	4	11	16.9%
	5	14	21.5%
	6	14	21.5%
	7	6	9.2%
	8	2	3.1%
CLS	Present	39	21.7%
	Absent	141	78.3%
Total tissue area (n=39) (mean [SD])		2.5 [0.9]	
CLS Density (n=39) (mean [SD])		12.0 [33.1]	

[1] The numbers on the pictograph indicate different body sizes characterised by increasing body weight with 1 being the slimmest and 9 the heaviest (Appendix 2).

Table 3: Association between CLS presence and BMI

BMI Category	CLS		P value
	Absent	**Present**	
Normal	25 (17.7%)	3 (7.7%)	0.220
Overweight	52 (36.9%)	13 (33.3%)	
Obese	64 (45.4%)	23 (59.0%)	

Table 4: Association between CLS density and BMI

	BMI Categories		
	Normal **18.5-24.9**	**Overweight** **25.0-29.9**	**Obese** **>30**
CLS Density (n=39) (median [IQR])	1.8 (1.0, 4.4)	1.9(0.4-7.7)	1.0(0.5,5.4)

Table 5: Binomial logistic regression model adjusted for age, ER status, and menopause

BMI Categories	**Odds Ratio**	**95% Confidence Interval**	**P value**
Normal	1.00	Reference	
Overweight	1.24	0.40 – 3.85	0.713
Obese	2.03	0.68 – 6.02	0.204

Table 6: Association between CLS and clinical and demographic characteristics

Characteristic		CLS		P Value
		Absent	Present	
Age (years) (median [IQR])		53.0 [44.0, 61.0]	53.0 [48.0, 58.0]	0.313
Menopause Status	Pre	53 (39.8%)	13 (34.2%)	0.669
	Peri	1 (0.8%)	0 (0.0%)	
	Post	79 (59.4%)	25 (65.8%)	
Survival Status	Alive	35 (97.2%)	11 (100.0%)	1.000
	Died	1 (2.8%)	0 (0.0%)	
Histological Grade	1	4 (3.0%)	2 (5.3%)	0.504
	2	72 (53.3%)	17 (44.7%)	
	3	59 (43.7%)	19 (50.0%)	
Pictograph (n=65)[2]	2	4 (9.5%)	1 94.3%)	0.344
	3	7 (16.7%)	6 (26.1%)	
	4	9 (21.4%)	2 (8.7%)	
	5	9 (21.4%)	5 (21.7%)	
	6	8 (19.0%)	6 (26.1%)	
	7	5 (11.9%)	1 (4.3%)	
	8	0 (0.0%)	2 (8.7%)	
Waist Circumference (n=50)		38.0 [33.0, 59.0]	46.0 [40.0, 68.0]	0.056
Hip Circumference (n=46)		46.5 [42.0, 52.0]	48.5 [46.0, 68.5]	0.137
Waist Hip Ratio (n=46)		0.85 [0.77, 0.92]	0.84 [0.80, 0.91]	0.965
Tumour size (n=172)		4.0 [2.0, 4.0]	4.0 [2.0, 4.0]	0.865
Stage (n=170)	1	22 (16.7%)	7 (18.4%)	0.456
	2	82 (62.1%)	27 (71.1%)	
	3	24 (18.2%)	3 (7.9%)	
	4	4 (3.0%)	1 (2.6%)	

[2] The numbers on the pictograph indicate different body sizes characterised by increasing body weight with 1 being the slimmest and 9 the heaviest (Appendix 2).

CHAPTER 4: DISCUSSION

Breast cancer (BC) is the most common malignancy worldwide including in Kenya where the incidence rate is estimated to be 16.1% (Harbeck et al., 2019). BC is a heterogeneous disease at the morphological and molecular level (Harbeck et al., 2019). The WHO lists up to 21 histological types, with invasive carcinoma of no special type being the most common worldwide (Michael Thun, 2017; Organisation, 2012). Most of the patients (82.2%) sampled in this study presented with invasive ductal carcinoma of no special type.

At the molecular level BC is classified into 4 intrinsic subtypes; luminal A, luminal B, HER-2 enriched, basal like subtypes (Perou et al., 2000). With an immunohistochemistry panel of ER, PR, HER2, Ki67, CK5/6, EGFR one can approximate the molecular subtypes of BC as determined by gene expression profiling (Schnitt, 2010). We found that 66.3% of the patients had Luminal A/B subtype similar to a study conducted by Sayed et al where 70.2% of patients(n=846) were Luminal A/B (Sayed et al., 2018).

Risk factors of BC include sex, age, estrogen, family history, gene mutations and obesity (Kashyap, Pal, Sharma, Koundal, & Belay, 2022; Sun, Zhao, Yang, Yao, & Zhu, 2017). The mean age and median age was 53 years. Of the patients sampled in the study, 39.4% were below 50 years, consistent with other studies which show that African patients present with BC earlier than other races (Abdulrahman & Rahman, 2012; Aziz et al., 1999; Lukong et al., 2017).

Obesity, a known risk factor for BC, is commonly measured as BMI (Harbeck et al., 2019; WHO, 2000). There has been an increase in obesity globally including Africa (Adeboye et al., 2012). Estimates from Kenya show that 20.5% and 9.1% of women aged 15-49 years are overweight and obese respectively (Mkuu et al., 2018). The link between BC and obesity is complex however certain hypothesis include the role of obesity related inflammation with formation of crown like structures are formed (Faria et al., 2020; Iyengar, Gucalp, et al., 2016).

Blair et al in an evaluation of 859 patients with BC showed that women who presented with tumors greater than 5 cm were more likely to have a BMI > 25kg/m2 (22.1%, n=859) compared to those with normal BMI (9%, n=859) (Blair et al., 2019). Obese women also had a 1.7-fold increased risk of stage 3 or 4 disease from the same study (Blair et al., 2019) A recent local study by Sayed et al showed that 68.7% of Kenyan BC patients(n=823) had a BMI >25kg/m2 (Sayed et al., 2018). Our study showed similar results as 48.3% of the patients were obese and 36.1% were overweight.

The link between obesity and BC development and progression is not clear. One of the mechanisms is that higher BMI is associated with adipocyte hypertrophy, adipocyte necrosis with formation of CLS. The activated macrophages within the CLS phagocytose the fat and secrete cytokines that maintain the inflammatory state. They also produce reactive oxygen species and reactive nitrogen species which are mutagenic (Berger, 2017; Hotamisligil, 2017). There is also increased expression of the NFkB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway which promotes synthesis of various cytokines(Zhu et al., 2020). Aromatase expression and activity is also increased within the adipose tissue resulting to elevated estrogen (Morris et al., 2011). All these contribute to the development and progression of BC (Faria et al., 2020; Iyengar, Gucalp, et al., 2016).

CLS was detected in surrounding non-tumour breast adipose tissue in 21.67% of cases. In multivariable logistic regression models, obese patients were approximately two-fold more likely to have CLS in their tissues than normal weight patients (Odds ratio (95% CI) = 2.03 (0.68-6.02)) but differences were not statistically significant ($p = 0.204$). This is consistent with previous studies which show a higher CLS prevalence in surrounding non-tumour breast adipose tissue of obese than normal weight women.

Observed differences were, however, not statistically significant, which could be due to the low prevalence of CLS in this population, the relatively small sample size, or due to inherent tumour characteristics within this population.

We also proffer that due to its low prevalence within this population, CLS may not be the ideal marker of white adipose tissue inflammation. Further studies looking in to macrophage density within surrounding breast tissue and the different types of macrophages are warranted.

CLS evaluation can be performed via various methods. We used CD68 immunohistochemistry consistent with other studies (Greenlee et al., 2019; Maliniak et al., 2020). However, Koru-Sengul et al used three different macrophage markers (CD163, CD206, CD40) and correlation was done with CD40 which highlights specifically M1- pro-inflammatory macrophages. This could explain for some of the discrepancies within our study. Moreover, tumour biology may have an effect as well given that there is a complex relationship between tumour and surrounding breast stroma (Hotamisligil, 2017).

Waist circumference (WC) measures central adiposity which is an important factor in obesity-related health risk(Ross et al., 2020). A larger WC is associated with a higher risk of both pre

and post-menopausal BC (Gaudet et al., 2014; Houghton et al., 2021). From our study, waist circumference showed a closer association with CLS (p=0.056) though the data was insufficient to draw statistically significant conclusions.

CLS detection rate was slightly lower than other studies (Greenlee et al., 2019; Zhao et al., 2020). This could have resulted from the use of one tissue block per case. Green lee et al (Greenlee et al., 2019) and Zhao et al (Zhao et al., 2020) both analysed multiple levels of the slides each approximately 50 micrometers apart. This could also account for our low detection rate as we only evaluated for CLS at one level. Future studies could use more surrounding breast tissue to increase the rate of detection.

However, consistent with other studies we found that most BC patients had higher BMI with 48.3% being obese (Neuhouser, Aragaki, et al., 2015; Reeves et al., 2007; White et al., 2015). This confirms the existence of a link between obesity and BC. The exact mechanisms for this may not be clear. It could be attributed to a complex interplay between breast adipose tissue, circulating adipokines and other systemic factors (Calle & Kaaks, 2004).

CLS were also present in normal weight patients. Iyengar et al and Zhao et al found CLS within 39% (n=72) and 31.9% (n=136) of women with normal BMI respectively (Iyengar et al., 2017).CLS within normal women could be attributed to a possible occurrence of a subclinical inflammatory state in the surrounding breast tissue or a systemic metabolic obese state in patients with normal BMI (Iyengar et al., 2017).

Iyengar et al (Iyengar et al., 2015) in an evaluation of 237 patients showed that the presence of CLS-B was positively associated with menopausal status (P=0.008) and BMI (P<0.001). We also found that patients with CLS were more likely to be postmenopausal than pre-menopausal (64% vs 36%). However, this difference was not statistically significant (p=0.92).

We also did not find any statistically significant association between CLS presence and density with BC risk factors specifically, age, menopausal status, subtypes of BC and overall survival among BC patients. Data on association between CLS and other clinical factors are infrequent. CLS are also seen in older patients compared to younger patients(Greenlee et al., 2019; Iyengar et al., 2015). We found that most patients (76.92%) with CLS were between 41-60 years. Age was however not statistically significantly associated with presence of CLS in this study (p=0.313).

CLS have been associated with reduced survival and shortened distant recurrence free survival in those who develop metastases in BC (Iyengar, Zhou, et al., 2016).We did not find a significant association between CLS presence with survival (p=1.00, n=47). Of note, we had scarce survival data for most of the patients.

This study has various benefits. To the best of our knowledge it is the only study in Kenya that has tried to understand the link between CLS, BMI and BC. It points to a possible association between increasing BMI and risk of BC. It serves as a stepping stone for additional studies to be done within this field to enhance knowledge of the relationship between BMI and BC with a special focus on tumour microenvironment.

4.1 Limitations

We encountered certain limitations while conducting the study. Firstly, our study is limited by its retrospective design of evaluation of white adipose tissue inflammation and BMI in BC. Secondly, we did not analyse CLS presence and density within a non-BC population. Therefore, a direct causation between white adipose tissue inflammation and BC causation cannot be made. Thirdly, we had a relatively small sample size which might have contributed to the nature of our findings. Furthermore, analysis was done on only one tissue block and on one microscopic level per case and this could have led to a low detection rate. We also had missing data for some of the variables hence it was difficult to efficiently determine the relationship with CLS presence. Other studies have used CD68 (Greenlee et al., 2019; Maliniak et al., 2020)to highlight macrophages forming CLS but it does not distinguish between the anti-inflammatory and pro-inflammatory macrophage types which could have different associations with risk factors.

CHAPTER 5: CONCLUSION

The findings were suggestive of higher CLS prevalence in surrounding non-tumour breast adipose tissue of obese than normal weight women with BC. Observed differences were, however, not statistically significant, which could be due to the low prevalence of CLS in this population, the relatively small sample size, or due to inherent tumour characteristics within this population. Due to its low prevalence, CLS may also not be the most ideal marker for white adipose tissue inflammation and further studies looking into macrophage density and different types of macrophages within surrounding breast tissue are recommended.

CHAPTER 6: RECOMMENDATIONS

Future studies could aim to use more than one tissue block and/or multiple microscopic levels could improve CLS detection. Prospectively designed studies with a larger sample size including a non-BC control population could be done to enhance understanding of the link between CLS, BC and BMI. Evaluation of CLS presence, macrophage density and the different types of macrophages within surrounding non-tumour breast tissue within BC patients should be carried out to better understand this tumour microenvironment. Further studies involving normal weight patients with inclusion of lipid profile, blood glucose, leptin, and breast aromatase levels and anti-inflammatory markers could help understand the link between BC and CLS in normal weight women.

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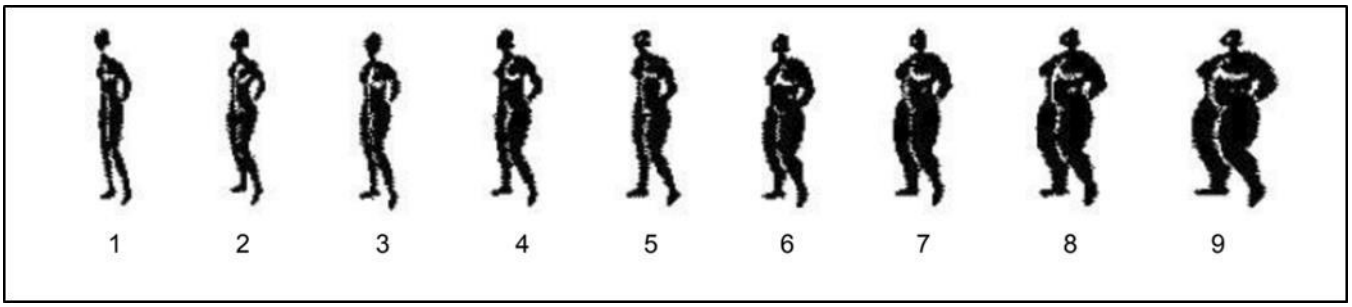
APPENDICES

APPENDIX 1: DATA COLLECTION SHEET

CHARACTERISTIC		CLS present	CLS absent	CLS low	CLS high
AGE in years	20-39				
	40-49				
	50-59				
	60-79				
BMI					
Underweight					
Normal					
Overweight					
Obese					
Histologic type					
IDC					
ILC					
Other					
Molecular subtype					
Luminal					
Basal like					
HER2-enriched					
Menopause					
Premenopausal					
Postmenopausal					
Histological grade					
Grade 1					
Grade 2					
Grade3					

APPENDIX 2: PICTOGRAPH CHART



[3]

[3] The pictograph chart shows different body sizes characterised by increasing body weight ranging from 1 to 9; 1 being the slimmest and 9 the heaviest.